Assessment of iron and vitamin A status in settings of inflammation: The BRINDA Project

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Declaración de Intereses:

No tengo ningún interés que declarar
If you can not measure it, you can not improve it.

~ Lord Kelvin
Objectives

- Provide a brief overview of the effects of inflammation on nutrient biomarkers, focusing on iron and vitamin A
- Review inflammation adjustment approaches
- Highlight key results from the BRINDA project
- Discuss programmatic and research implications
Nutritional status

Infection / Inflammation

Stress

Tissue damage

Infection

Inflammation

Inflammatory cytokines
- Interleukin 1
- Interleukin 6
- Tumor necrosis factor

Acute phase proteins
- C-reactive protein (CRP)
- α-1 acid glycoprotein (AGP)
- Retinol binding protein (RBP)
- Ferritin
How infection/inflammation affect micronutrient status

Adapted from Bresnahan, *Adv Nutr* 2014
Need to measure both CRP and AGP

Reference
(normal CRP + AGP)

Incubation
(elevated CRP)

Early convalescence
(elevated CRP + AGP)

Late convalescence
(elevated AGP)

Ann Clin Biochem 2008
Many Nutrient Biomarkers are Affected by Inflammation

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Magnitude and Direction of Inflammation Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iron</strong></td>
<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td>+++</td>
</tr>
<tr>
<td>Transferrin receptor (TfR)</td>
<td>+</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>-</td>
</tr>
<tr>
<td>Hepcidin</td>
<td>++</td>
</tr>
<tr>
<td><strong>Vitamin A</strong></td>
<td></td>
</tr>
<tr>
<td>Retinol; Retinol binding protein (RBP)</td>
<td>--</td>
</tr>
<tr>
<td><strong>Serum zinc</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Folate and vitamin B12</strong></td>
<td>?</td>
</tr>
</tbody>
</table>

INSPIRE report, *JN.* 2015
Why do we care about the effects of inflammation on micronutrient biomarkers?

The use of nutrition biomarkers in inflamed individuals can lead to spurious conclusions:

- Incorrect diagnosis of *individuals*
- Over or under-estimation of prevalence of micronutrient deficiency in *populations*
- May interfere with ability to accurately assess impact of micronutrient interventions
Hypothetical changes in ferritin concentrations in response to inflammation

- **Ferritin µg/L**
  - **A**
  - **B**
  - **C**

- **Deficient**
- **Sufficient**

- **Time**
- **Usual ferritin level**
- **Temporary rise in ferritin**
- **Return to usual ferritin level**

- **Inflammatory Event**

- **Individual temporarily classified as sufficient**

Hypothetical changes in ferritin concentrations in response to inflammation.
Current Recommendations for Iron and Vitamin A Assessment in the Setting of Inflammation

- Perform survey in season of low inflammation
- Measure acute phase protein and exclude individuals with elevated CRP or AGP
- For inflamed individuals, use higher ferritin cutoff (<30 ug/L)

"Clinical and subclinical infections may also lower serum retinol concentrations by as much as 25%, making knowledge of the infection burden of the population critical for accurately interpreting the serum retinol distribution."

Guidance: None
Available approaches to account for inflammation

1. Ignore inflammation
2. Exclude individuals with inflammation
3. Adjust nutrient biomarker cut-off (ferritin < 30)
4. Apply fixed correction factor*
5. Apply regression correction
6. Other/Combination of above

* No consensus on preferred method

* Thurnham, J Nutr 2015
Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia (BRINDA)

Goal → Refine approaches to estimate nutritional status

Why? → Improve the targeting, design and effectiveness of nutrition programs

How? → Pool high quality nutrition survey data

Inclusion criteria:
- Household-based
- National or regionally representative
- CRP or AGP + nutrient biomarker

Total of 25 surveys:
- n = 36k (PSC), 52k (WRA), 39k (SAC)
Biomarkers measured in preschool children country surveys

- Hemoglobin
- CRP
- AGP
- Ferritin
- Retinol/RBP
- Zinc
- B12
- Serum folate
- RBC folate
- Malaria
Laboratory methodology in BRINDA surveys

- **Hemoglobin**
  - 21 out of 25 surveys used Hemocue

- **CRP, AGP**
  - 12 used Erhardt ELISA*

- **Zinc**
  - 8 out of 13 used atomic spectrometry

- **Folate and B12**
  - 4 microbiological assay, 8 immunoassay, 3 radioassay

- **Malaria in 8 surveys**

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*Sandwich ELISA
- CRP
- AGP
- Ferritin
- sTfR
- RBP
Analytical approach

- Data cleaning, harmonization and quality control
- Individual survey and target-group specific analysis, accounting for cluster design and sampling weights
- Pooled meta-analysis using individual and country-level variables, stratified by region, malaria, inflammation prevalence
Key findings

Prevalence of inflammation in preschool children

Any inflammation = 48%

Inflammation is common globally and direct measures of CRP and AGP are needed

Merrill et al. AJCN, 2017
Distribution of CRP for preschool children

Inflamed?

1st decile (0.10 mg/L)

CRP = 5 mg/L

Inflamed!

Inflamed?

n=36,120
Prevalence of low ferritin by AGP decile, children

AGP ≤ 1.0  AGP > 1.0

% ferritin < 12 µg/L

lowest inflammation  highest inflammation

n=12,279
Effects of CRP and AGP on nutrient biomarkers are linear, especially in children

![Graph showing the effects of CRP and AGP on nutrient biomarkers.](image)

- **CRP DECILE (FROM LOWEST TO HIGHEST)**
  - ferritin
  - sTfR
  - RBP

- **AGP DECILE (FROM LOWEST TO HIGHEST)**
  - ferritin
  - sTfR
  - RBP

n=9000 to 28000
Adjusted ferritin = ferritin - $\beta_1(\text{AGP}_{\text{obs}} - \text{AGP}_{\text{ref}})$ - $\beta_2(\text{CRP}_{\text{obs}} - \text{CRP}_{\text{ref}})$
Vitamin A deficiency likely overestimated if ignore inflammation

Larson, et al. *AJCN* 2017 & *Nutrients* 2018
### Summary of change in ID and VAD prevalence by inflammation adjustment method, children

<table>
<thead>
<tr>
<th>Method</th>
<th>Ferritin</th>
<th>TfR</th>
<th>RBP</th>
<th>Retinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exclusion¹,²</td>
<td>+ 6.5¹</td>
<td>- 6.5²</td>
<td>- 12.0</td>
<td>- 6.6</td>
</tr>
<tr>
<td>Correction factor</td>
<td>+ 6.5</td>
<td>- 6.0</td>
<td>- 10.1</td>
<td>- 4.5</td>
</tr>
<tr>
<td>Regression correction</td>
<td>+ 24.7</td>
<td>- 14.6</td>
<td>- 16.4</td>
<td>- 13.1</td>
</tr>
</tbody>
</table>

¹Exclude based on CRP>5 mg/L or AGP >1 g/L; ²Exclude based on AGP > 1g/L

Based on countries that had both CRP and AGP, compared to no adjustment.
What about “low inflammation” settings?

**US and Latin America**

<table>
<thead>
<tr>
<th>Method</th>
<th>Median percentage point difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ferritin</td>
</tr>
<tr>
<td>Regression correction</td>
<td>+ 6.9</td>
</tr>
</tbody>
</table>

Surveys included: US, Colombia, Ecuador, Mexico, Nicaragua

None of the surveys measured both CRP and AGP!
Summary of Key BRINDA Questions and Findings

1. Do we need to measure biomarkers of inflammation when assessing iron or vitamin A status?
   - Yes, even in “low inflammation” settings

2. Do we need to measure more than 1 inflammation biomarker (CRP and/or AGP)?
   - Yes

3. What approach should be used to adjust for inflammation?
   - Regression correction

4. Additional correction for malaria?
   - Unlikely when using regression correction

5. What prevalence estimates should be reported?
   - Both unadjusted and inflammation-adjusted estimates

Findings from the BRINDA project. SPRING, 2017
Key successes to date

- Multi-country and institution collaborative group
- Large-scale dissemination
  - 14 scientific papers
  - SPRING programmatic brief
  - Global nutrition symposia, website
- Inform global nutrition policy
- Phase 2 complete and starting phase 3
- Foundation for future activities....

www.BRINDA-nutrition.org
BRINDA 3 and beyond

- Continued dissemination and feedback
  - Web-based tutorials, scientific publications, seminars, webinars
  - Analytical tools

- Publically share BRINDA data and add new data

- New research questions
  - Population groups (pregnancy, mother-child dyads, adolescents)
  - Other micronutrients (vitamin D), inflammation biomarkers (IL-6)
  - Improve global anemia estimates
  - Applicability in clinical settings, program evaluations
BRINDA Working Group Members

- **Steering Committee**
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  - Rafael Flores (CDC)
  - Sorrel Namaste (ICF, DHS Program)
  - Dan Raiten (NICHD)
  - Lynette Neufeld (GAIN)
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  - Emma Yu
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- **Country Representatives**

- **Other collaborators**
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The findings and conclusions in this presentation have not been formally disseminated by CDC, GAIN, NIH, or USAID and should not be construed to represent any agency determination or policy.
Gracias!

For more information:
BRINDA-nutrition.org
When to apply the BRINDA Correction Approach

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Not Recommended</th>
</tr>
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<tbody>
<tr>
<td>Serum ferritin (WRA/PSC)</td>
<td>Retinol binding protein (WRA)</td>
</tr>
<tr>
<td>- CRP and AGP</td>
<td>Serum zinc (WRA)</td>
</tr>
<tr>
<td>Retinol binding protein and retinol (PSC)</td>
<td>Serum folate (PSC/WRA)</td>
</tr>
<tr>
<td>- CRP and AGP</td>
<td>RBC folate (PSC/WRA)</td>
</tr>
<tr>
<td>sTfR (WRA/PSC)</td>
<td>Vitamin B12 (PSC/WRA)</td>
</tr>
<tr>
<td>- AGP only</td>
<td></td>
</tr>
<tr>
<td>Serum zinc (PSC)</td>
<td></td>
</tr>
<tr>
<td>- CRP and AGP</td>
<td></td>
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</tbody>
</table>

PSC= preschool age children  
WRA= women of reproductive (non-pregnant)

[www.BRINDA-nutrition.org](http://www.BRINDA-nutrition.org)
ADDITIONAL REFERENCE SLIDES
Key findings

- Ignoring inflammation
  - **underestimates** prevalence of iron deficiency by median of 25% points
  - **overestimates** prevalence of vitamin A deficiency by 16% points

- Regression correction approach should be used and both inflammation-adjusted and unadjusted estimates of deficiency should be reported

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Findings from the BRINDA project. SPRING, 2017
Feasibility of Regression Correction

SAS Macro developed

Example of excerpt from SAS macro
/* This macro adjusts a given biomarker for C-reactive protein, Alpha-1-acid glycoprotein, and malaria under a simple sampling design. The macro requires name of the data set, and the names of the ID, CRP, AGP, malaria and biomarker variables.*/

%macro BiomarkerChildAdj(dataset, id, crp, agp, malaria, biomarker);

Plan to develop software similar to WHO anthro

Software for assessing growth and development of the world's children
Proportion of anemia associated with iron deficiency by country infection burden

Preschool Children

- Very high infection burden:
  - Anemia: 68%
  - IDA: 39%
  - ID: 54%

- High infection burden:
  - Anemia: 57%
  - IDA: 31%
  - ID: 44%

- Medium infection burden:
  - Anemia: 18%
  - IDA: 5%
  - ID: 23%

- Low infection burden:
  - Anemia: 2%
  - IDA: 1%
  - ID: 13%

% anemic with ID: 58%
Population Attrib Fraction: 8%

Women of reproductive age

- High infection burden:
  - Anemia: 41%
  - IDA: 14%
  - ID: 22%

- Medium infection burden:
  - Anemia: 12%
  - IDA: 8%
  - ID: 33%

- Low infection burden:
  - Anemia: 7%
  - IDA: 5%
  - ID: 19%

% anemic with ID: 35%

AJCN, 2017